(19) JAPANESE PATENT OFFICE (JP)
(12) Official Gazette for Laid-Open Patent Applications (A)
(11) Japanese Laid-Open Patent Application (Kokai) No. 5-320061
(43) Laying-Open Date:3 December 1993

(51)	Int.Cl.[5]	Ident. Symbols		Internal Office Nos.	FI	Technology Designation
A61K	35/78	ADD	J	7180-4C		
		ACL		7180-4C		
		ADA		7180-4C		
		AED		7180-4C		
	7/00		K	9165-4C		
A61K	7/00		X	9165-4C		
			Y	9165-4C		
	7/48			9051-4C		
C09K	15/34					

Request for Examination: Not yet requested Number of Claims: 1 (Total of 9 pages)
Continued on last page

(21) Application No.: 4-150155

(22) Application Date: 19 May 1991

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(54) Title of the Invention: An Active Oxygen Elimination Agent [derived] from Legumes

(57) [Abstract]

[Objective] To provide an active oxygen elimination agent, using legumes as the raw materials, that is safe and inexpensive and that can be used in broad range of fields such as medicinal drugs, food products and cosmetic products.

[Structure] An active oxygen elimination agent characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[Claims]

[Claim 1] An active oxygen elimination agent characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to an active oxygen elimination agent that is safe and inexpensive and that can be used in a broad range of fields such as medicinal drugs, food products and cosmetic products characterized in that it is an aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them.

[0002]

[Prior art] When a person has a healthy body, the active oxygen in the body and SOD (superoxide dismutase), which is the active oxygen elimination enzyme in the body are usually in balance and the concentration of active oxygen is maintained at an essentially fixed level. However, production of SOD is decreased due to dietary imbalance, excess stress and aging. On the other hand, active oxygen is increased by smoking and air pollution.

[0003] As a result, active oxygen is present in the body in excess and brings about various kinds of tissue damage. Among the elderly in particular, impairments such as rheumatoid arthritis and Paget's disease are brought about as a result of a decrease in SOD activity and increase in active oxygen concentrations. In addition, the lipid peroxidases that are produced by active oxygen are the principal causes of such modern diseases as myocardial infarction, stroke, cataract, liver spots, freckles, wrinkles, diabetes mellitus, arteriosclerosis, stiffness of the shoulders and sensitivity to cold.

[0004] In the elderly, active oxygen is particularly readily produced in sites such as the skin that are directly affected by environmental factors such as ultraviolet rays. For this reason, production of the pigment melanin and lesions such as liver spots and small wrinkles readily tend to occur accompanying an increase in active oxygen concentration.

[0005] Accordingly, attention has been drawn to SOD, which eliminates the excess active oxygen that is the basis of the various lesions described above and attempts have been made to produce SOD as a medicinal drug product and to add it to cosmetic products and food products for the purpose of preventing or treating these lesions. However, because SOD is unstable in the presence of heat and is deactivated when it is administered orally, and, further, because it is expensive, success has not yet been achieved in elimination of active oxygen by means of SOD.

[0006] On the basis of the circumstances described above, research was conducted on active oxygen elimination agents (substances that contain antioxidants that act in the same way as the enzyme SOD). An active oxygen elimination agent based on raw drug extracts was developed. However, it is made of special raw materials and is expensive. At present, it has not been possible to supply a stable substance.

[0007]

[Problems the invention is intended to solve] Since the time that various lesions attributable to active oxygen as described above were found, there has been a great deal of research with the objective of eliminating active oxygen in the body. Facing an aging society as we are, there is the desire for people to pass their old age in a healthy state. Attention has also been draw to active oxygen elimination agents from a cosmetic standpoint.

[0008] Therefore, there is the desire for the development of an active oxygen elimination agent that is safe for human subjects, that is inexpensive, that is of superior effectiveness in the elimination of active oxygen that brings about various lesions, that can be manufactured simply and for which there can be a stable supply.

[0009]

[Means for solving the problems] The inventors conducted research on various plant components from the standpoint of the harmonization of plants and animals. In the course of this research, it was ascertained that there were many possibilities and effects that had not been anticipated up to the present in legumes, as represented by soy beans, peas, kidney beans, red beans and fava beans. Accordingly, we took up as our research theme the legumes, which have been used as food throughout the world for a long time and which have been demonstrated to be of the highest safety, and conducted research on the overall utilization of legumes. One of these themes was to conduct intensive research on an oxygen elimination agents derived from legumes. When determinations were made of the active oxygen elimination effectiveness of extracts or juices of legumes in unaltered form or of substances that contained them, it was ascertained that there was an extremely marked active oxygen elimination effect. This resulted in the perfection of this invention.

[0010] Specifically, this invention is an active oxygen elimination agent characterized in that it is a aqueous extract or an organic solvent extract from legumes or a juice from fresh legumes in unaltered form or in that it contains them. By squeezing legumes, making aqueous extracts (including acid and alkali extraction) or by extraction with organic solvents such as alcohol, oxygen elimination agents that are simple, inexpensive and completely safe and that have excellent effectiveness as described above can be obtained. The legumes that are used as raw materials may be soy beans, peas, kidney beans, red beans and fava beans. Further, the legumes may be immature fresh legumes, completely mature fresh legumes or mature legumes that have been treated by drying.

[0011] When the legumes are made as aqueous extracts or organic solvent extracts, surface area is increased when the legumes are first pulverized or made into powders, for which reason there is extremely good extraction efficiency. The general method is to use a pulverizer. However, they may also be used without pulverizing them. In this case, a long time is necessary for decomposition and extraction of the legume tissues.

[0012] In aqueous extraction, the legumes may be left in unaltered form, or, preferably, they are pulverized and made into a powder and water is added. Extraction can be performed efficiently with a quantity of water added of 2 to 5 times the volume of the legumes. However, the quantity may be selected appropriately depending on yield, workability and the final objective of use. Following that, the materials are heated and extraction is completed at the point in time that a state of boiling is reached. After extraction has been completed, a clear extract is obtained when squeezing or filtering is performed, depending on the objective of use. Extraction may also be performed by adding boiling water at the outset.

[0013] Although the effective component in the extract solution has not been clarified, it has been confirmed that this unknown component is stable in the presence of heat. Therefore, the extraction temperature can be high and thus is efficient. Extraction can be performed sufficiently if the materials are allowed to stand for a long time. However, at temperatures below 40°C, it is necessary to make the pH either acidic or alkaline or to add a preservative. Extraction time may be several minutes in extraction by boiling. At lower intermediate temperatures, several hours to twenty-four hours is necessary.

At low temperatures, several days to one month is necessary due to the pulverized state of the legumes. However, in this case, heating as much as possible is finally more effective.

[0014] The greatest problem in aqueous extraction of legumes in which the principal component is starch is the phenomenon of gelatinization. If gelatinization occurs, there is poor extraction efficiency and there are extremely great difficulties in practical operations. An efficient extraction method in aqueous extraction is to perform a pretreatment with an acid or alkali or to perform a pretreatment by reacting an enzyme that acts on the tissues of the legume (for example, cellulase or lipase). The reason for doing this is thought to be that the effective component is made more easily extractable by the pretreatment.

[0016] It was also ascertained that extracts having this effect are processed in organic solvent extraction as well. In addition to advancing clarification of the effective component, this is also extremely effective for such uses as extracting the effective component under difficult circumstances and in compounding it with substances that are not soluble in water. In this case, it is preferable for it to be finely pulverized as much as possible or to be made into a powder. It is desirable that the organic solvents that are used here be substances such as alcohol that are safe when administered to human beings.

[0017] Squeezing out the juice of legumes has been effective for collection of the effective component from legumes. When juice is squeezed from legumes, ordinary methods may be used such as grinding immature fresh legumes, mature legumes or legumes that have been subjected to heat treatment, enclosing them in a cloth bag and pressing them or squeezing them using a squeezing machine. In this case as well, it has been ascertained that there is an active oxygen elimination action, although the effect is weak, as a result of further subjecting the residue to aqueous extraction or organic solvent extraction after squeezing has been completed.

[0018] There are equivalent effects when fermentation such as alcohol fermentation or lactic acid fermentation is used in combination. Extraction of the product of this invention from legumes is more effective when organic solvent extraction or aqueous extraction is performed as described above and when the effective component in the extract is further subjected to solvent extraction. However, considering that the product is obtained in a concentrated state in this process, equivalent effects can be obtained by concentration.

[0019] Sugar and dextrin are present in the juices and extracted material. Because they are viscous, there are difficulties in obtaining effects depending on the intended use. In this case, the sugar may be removed by having it consumed by yeast, by fractionating the effective component with an adsorbent or by extraction with an organic solvent. In any case, effects appear when extraction is performed, and, depending on the intended use, the unnecessary components may be removed by various methods.

[0020] Legumes have been used every day as foods for a long time and they are so familiar to us that we may not have considered the concept of using then as active oxygen elimination agents. In addition to being eaten in their form as legumes, they are also used in various processed forms such as *tofu* [bean curd], dried bean curd, fried bean curd, bean jam, soybean flour, fermented soy beans, *miso* and soy sauce. However, the concept of and method for extraction of legumes have not been adopted. We believe that the reason for this is that starch legumes become gelatinous when extraction is performed by heating and that, conventionally, extraction was thought to be extremely difficult. For this reason, in this invention the objective can be achieved by facilitating extraction by the action of amylase when organic solvent extraction, acid or alkali extraction or aqueous extraction is performed.

[0021] In the case of legumes such as soybeans in which the principal components are proteins, extraction has been performed conventionally using a method in which soybean milk is collected. However, this is used solely as a nutritional source and has not been used as an active oxygen elimination agent. In addition, essentially no active oxygen elimination action has been found for soybean milk. Specifically, it appears that the objective can be obtained by performing treatment with enzymes such as cellulase and lipase that act on the tissues of legumes or treatments using acids or alkalis as pretreatments. Thus, the effective components can be extracted as superior active oxygen elimination agents by performing sufficient extraction operations on the raw materials.

[0022] We shall now describe the active oxygen elimination effect of the products of this invention. First, we investigated their effects as superoxide elimination agents by various potato operational methods. The test method used was the NBT method.

[0023] Preparation of reagents

1) 0.05 M Na_2CO_3 buffer solution (pH 10.2)

2) 3 mM xanthine solution; 45.64 mg of xanthine was dissolved in the buffer solution to make 100 ml.

3) 3 mM EDTA solution; 111.7 mg of EDTA·2Na was dissolved in distilled water to make 100 ml

4) BSA solution; 15 mg of Bovine Serum Albumin (manufactured by Sigma) was dissolved
in distilled water to make 10 ml.

5) 0.75 mM NBT solution; 61.32 mg of NBT (nitroblue tetrazolium) was dissolved in distilled water to make 100 ml.

6) Xanthine oxidase solution; Xanthine oxidase was diluted with distilled water and its absorbance in a blank test by an operational method (analytical method) to be described subsequently was adjusted to be in the range of 0.2 to 23.

7) 6 mM $CuCl_2$ solution; 102.29 mg of $CuCl_2 \cdot 2H_2O$ was dissolved in distilled water to make 100 ml.

[0024] Operational methods

1) 2.4 ml of Na_2CO_3 buffer solution was collected in a test tube and amounts of 0.1 ml of xanthine solution, EDTA solution, BSA solution and NBT solution were added to it.

2) Next, 0.1 ml of reagent solution was added and the mixture was allowed to stand for 10 minutes at 0°C, after which 0.1 ml of xanthine oxidase solution was added, the mixture was stirred rapidly and was then incubated at 25°C.

3) After 20 minutes, 0.1 ml of $CuCl_2$ solution was added to stop the reaction and absorbance was determined at 560 nm.

4) For the purpose of comparison, the same procedure was carried out on 0.1 ml of an aqueous solution of superoxide dismutase (Cu, Zn type SOD; activity of 3000 to 4000 units/mg; Wako Junyaku) in place of the sample. The value was expressed taking the superoxide elimination rate as 100.

5) The same procedure was carried out using distilled water in place of the sample as a blank. The results of the determinations are shown in Table 1.

[0025]

[Table 1]

	Red Beans		Broad beans		Soybeans		SOD
	Aqueous extraction	Organic solvent extraction	Aqueous extraction	Organic solvent extraction	Aqueous extraction	Organic solvent extraction	
Super oxidase (SO) elimination rate (%)	88	95	89	63	66	50	100

Note 1 The red bean extract used was the product of this invention that was obtained in Example 1.
Note 2 The soybean organic solvent extract used was the product of this invention that was obtained in Example 2.

[0026] As shown above, superoxide elimination effects were found for both the aqueous extracts and organic solvent extracts. It was further ascertained that these effects were essentially the same effects as when alcohol fermentation and lactic acid fermentation are performed.

[0027] Next, a study was conducted of the heat stability of the products of this invention. First, the products of this invention obtained in Example 1 and SOD were heated for 10 minutes at 90°C and the superoxide elimination rates were studied. Determination of the superoxide elimination rates was performed by the method described above. The results are shown in Table 2.

[0028]

[Table 2]

	Red bean aqueous extract	Broad bean aqueous extract	Soybean aqueous extract	SOD
SO elimination rate (%)	89	88	65	0

Note: The extracts used were those obtained in the respective examples.

[0029] As shown above, it was found that SOD was unstable in the presence of heat, whereas the products of this invention exhibited superior thermal stability. On the basis of this finding, it can be said that the effective component of the product of this invention that eliminates active oxygen is of superior stability in the presence of heat. Because the products of this invention exhibit marked active oxygen eliminating effects, and, moreover, are safe, they can be used in medicinal drugs, cosmetic products and food products. Next, we shall describe their uses.

[0030] As medicinal drug products, they can be used as antiulcerative agents. Next, we shall present the experimental methods whereby the antiulcerative action of the products of this invention is studied and the results of these experiments. A study was conducted of the action of products of this invention when administered orally in restrained water immersion stress ulcers. This was performed in accordance with the method of Watanabe et al.. Specifically, eight week old male ddY strain mice were fasted for 24 hours, after which 0.3 ml/mouse of the product of this invention obtained in Example 1 was administered orally. After 30 minutes, the mouse was placed in a stress cage and was immersed in water at 15°C up to the xiphoid process and was subjected to a restrained water immersion test. After 5 hours, the mouse was killed by dislocation of the cervical vertebrae and the stomach was excised. Following that, 1.5 ml of 1% formalin solution was injected into the stomach. In addition, the stomach tissues were gently fixed by immersion in this same solution and were allowed to stand for 24 hours. Following that, an incision was made along the greater curvature of the stomach and the lengths (mm) of the lesions that had been generated in the gastric glands region were determined. The sum per animal was expressed as the ulcerative coefficient. Animals given oral administration of the same quantity of physiological saline solution 30 minutes before introduction into stress cages were used as controls. Fifteen mice were used in each group. The results were summarized in Table 3.

[0031]

[Table 3]

	Dose (ml)/mouse	No. of samples	Avg. of ulcerative coefficients
Physiological saline solution	0.3	15	65.8
Product of this invention	0.3	15	34.9

[0032] As shown in Table 3, the average of the ulcerative coefficients in the mice that had been given physiological saline solution as a control was 65.8, whereas the average of ulcerative coefficients in mice to which the product of this invention had been administered was 34.9. Thus, it was clearly ascertained that the product of this invention was effective as an antiulcerative agent against restrained water immersion stress ulcers on oral administration. As a result, it was ascertained that products of this invention act directly from the gastric mucosa and exhibit an effective action as antiulcerative agents.

[0033] Next, products of this invention can be used as skin treatment agents. Products of this invention were applied twice a day, in the morning and evening, to a panel of patients suffering from various skin diseases, and these treatments were continued for one month. The diagnosed results are shown in Table 4.

[0034]

[Table 4]

	Marked Improvement	Useful	Somewhat Useful	Undecided Which	Discontinued	Usefulness (%)
Scratches, cuts	0	3	5	4	0	66.7
Burns	1	3	5	2	0	81.8
Diaper rash	0	3	6	2	0	81.8
Insect bites	0	1	4	2	0	71.4
Eruptions, pimples	0	5	9	4	0	77.8
Blackheads	2	3	5	2	0	83.3
Chapping, cracking	0	3	3	2	0	75.0
Xeroderma	1	2	8	2	0	84.6
Itching of skin	0	2	12	5	0	73.7
Eczema	1	4	6	3	0	78.6
Atopic dermatitis	1	5	4	6	0	62.5
Vesicular mycotic infections	0	3	4	1	0	87.5
Keratinized mycotic infections	0	4	2	1	0	85.7

(Note) 1 The product of this invention obtained in Example 1 was used.

(Note) 2 Usefulness is the overall percentage of marked improvement + useful + somewhat useful

(Note) 3 Evaluations were made by specialist physicians.

(Note) 4 The panel consisted of a total of 68 patients, 35 males and 33 females. The average age was 32.5 years (age 1 to 79).

[0035] As shown in Table 4 above, because this product was effected as a therapeutic agent for diverse skin conditions, it was concluded that is has a fibroblast activating action and that it also has an antimicrobial action. Further, because it was useful in xeroderma and blackheads, it was concluded that it has a humectant action and an action that inhibits increase of sebum to a suitable degree. The experimental method whereby this humectant action and action in inhibiting the increase of sebum to a suitable degree was studied and the results were as follows.

[0036] First, in order to illustrate the intensity of the humectant action of this invention, a single application experiment was performed using a moisture meter (SKICON 200). The determination conditions were an environment set to a room temperature of 20°C and a relative humidity of 65%. The members of the panel were allowed to rest in this environment for about 10 minutes before the determinations. The test sites that were selected were (bilateral) sites on the forearm on which exanthema was not found. The members of the panel were 5 individuals who were suffering from xeroderma. Figure 1 shows the average values for changes in the water content of the stratum corneum as read from the moisture meter in this experiment (in which the product of this invention obtained in Example 1 was used) and in the control experiment (in which water was used). The method of determination in the single application experiment is described below.

[0037] Determination method

1) Test sites and control sites of 5 × 5 cm were established on the forearms of the panel members.

2) The water content of the stratum corneum in these sites was determined.

3) Determinations were made of the water content of the stratum corneum immediately after application of the test material and after 30, 60, 90 and 120 minutes.

[0038] From Figure 1, it can be seen that the water content of the stratum corneum immediately after application of the product of this invention was approximately 8 times greater than that in the controls. When the findings from 30 minutes up to 120 minutes after application are examined, it can be seen that water content in the sites at which the product of this invention was applied were maintained at levels 2 to 3 times that in the controls up to 120 minutes.

[0039] Next, in order to provide numerical corroboration of the therapeutic effects of the product of this invention in xeroderma, water load experiments were performed using a moisture meter (SKICON 200) before use of the product of this invention and after two weeks of use. The panel consisted of the five individuals used for Figure 1 and the same determination conditions were used as in the single application experiments. A control group (in which determinations were made for sites at which the product of this invention was not applied) had to be established so that seasonal changes in the water content of the stratum corneum in vivo would not affect the evaluations of effectiveness. The water content of the stratum corneum was shown as an average value for the five panel members. The results are shown in Figure 2. The product of this invention that was used was the product obtained in Example 1. The method of determination in the water load experiment is described below.

[0040] Determination method

1) The water content of the stratum corneum was determined at the test site.

2) One drop of distilled water was placed on the test site and the water drop was completely wiped off with dry gauze after 10 seconds.

3) Water content of the stratum corneum was determined immediately after wiping and after 30, 60, 90 and 120 seconds.

[0041] As shown in the graph of Figure 2, improvement was found at the same time in the water absorption capacity of the skin (which was found by subtracting the value for water content of the stratum corneum before loading from the water content of the stratum corneum at

0 seconds after water loading) and in its water retaining capacity (the curve traced for water content of the stratum corneum from 0 seconds up to 120 seconds after water loading).

[0042] Specifically, before use of the product of this invention, the water content of the stratum corneum of the skin before water loading was extremely low (average of 4.6) and water absorption capacity (average of 42.0) was quite low. In addition, in the study of water retention capacity, the water absorbed in the stratum corneum of the skin of normal persons gradually decreased, with a return to values before water loading being seen 30 seconds after water loading. These results indicate that water absorption capacity, water retention capacity and barrier functions all decreased in the diseased stratum corneum for which determinations were made. By contrast, after use of the product of this invention, both the water content and the water absorption capacity of the stratum corneum of the skin increased to more than twice that before water loading and water retention capacity was also considerably improved so that it was essentially no different from that of normal individuals.

[0043] On the basis of these findings, it can be said that the product of this invention has a superior action in improving the state of water content and the barrier function of diseased stratum corneum. When the products of this invention are evaluated taking into consideration the humectant action obtained in the single application experiments, it can be said that the products of this invention increase the water absorption capacity and water retention capacity of the stratum corneum, that they absorb large quantities of water from the outside and that they have a humectant action that confers on the stratum corneum the property that it does not release water once it has been absorbed.

[0044] Further, in order to provide experimental verification of the inhibitory effect of products of this invention on secretion of sebum, determinations were made in changes in the quantity of sebum after washing of the face. The panel consisted of five individuals selected at random from the group used for Table 4. Figure 3 shows the average values for changes in the quantity of sebum in this experiment (application of the product of this invention after washing the face) and in the control experiment (washing of the face only). The product of this invention that was used was that obtained in Example 1.

[0045] As shown in the graph in Figure 3, when the product of this invention was applied, it was ascertained that an increase in the quantity of sebum was considerably inhibited. The prophylactic and therapeutic effects in blackheads were also supported by the inhibitory effects of the products of this invention on secretion of sebum. Further, when products of this invention were applied to the skin as cosmetic products, it was ascertained from the following experiments that there was a smoothing effect on the texture of the skin, that there is a wrinkle stretching rejuvenating effect and an aging preventing effect.

[0046] The product of this invention was applied twice a day for one month to sites on the right arms of the members of the panel and determinations of the sites of application of the product of this invention were made with a kinematic friction meter. The same sites on the left arm were used as controls. The panel consisted of six members.

The determination conditions are described below.

Temperature: 25°C
Humidity: 60°
Sensor used: KES-SE friction sensitivity tester SE-2 type (0.5 mm piano wire used)
Friction static load: 50 gf
Determination speed: 1 mm/sec
Determination distance: 30 mm (integrated effective range, 20 mm)

[0047] The MMD (coefficient of variation) for the sites on the left arm to which the product of this invention was not applied was 0.0186. However, the MMD (coefficient of variation) on the sites on the right arms to which the product of this invention had been applied for one month was less than 0.0084. The average values for the six subjects were essentially the same. It is thought that this was because there was little variation due to irregularities of the surfaces. From this finding, it was ascertained that the skin had become smoother and that there was stretching of wrinkles and rejuvenation. When MIU (coefficient of friction) was studied at the same time, it was 0.138 before application and less than 0.102 after application for one month. Thus, it was ascertained that there was a smoothing effect on the skin, a softening effect on the skin and that there was also an aging preventive effect.

[0048] In order to verify the beautifying effect of this invention, an experiment was conducted on its inhibitory action on tyrosinase. As the operational method, amounts of 1 ml each of substrate solution (0.04% tyrosine solution) and of buffer solution (McIlvaine Buffer, pH 6.8) were precisely collected in an absorption cell, amounts of 1 ml of water and of the product of this invention obtained in Example 1 were precisely introduced and the materials were mixed by stirring. After 5 minutes, the absorbance scale was zero corrected in alignment with a wavelength of 475 nm. Next, 0.02 ml of tyrosinase solution (obtained by dissolving 5.3 mg of tyrosinase in 0.9% NaCl solution) was precisely added. The mixture was immediately stirred and then incubated. The absorbance at that time was determined over time (at 3 minute intervals).

[0049]

[Table 5]

Time in Minutes	Water	Product of this Invention
0	0.011	0.012
3	0.058	0.045
6	0.152	0.059
9	0.243	0.097
12	0.316	0.099
15	0.414	0.118
18	0.498	0.133
21	0.552	0.142
24	0.621	0.151
27	0.623	0.157
30	0.629	0.162

[0050] From the results of the determinations shown in Table 5, it can be seen that there was a tyrosinase activity inhibiting action. On this basis, it can be said that the products of this invention have a beautifying action.

[0051] Further, as stated above, the products of this invention have a humectant action to the extent that they can be used as medicinal drug products. Consequently, they have actions that are basic for cosmetic products and have wide uses as creams, emulsions, toilet water, cleansers, packs and soaps. Effects similar to those described above can also be obtained by drinking the products of this invention.

[0052] The products of this invention can be used as preservatives of food products and as agents for maintaining freshness. We next conducted tests of the antibacterial activity of the products of this invention against Bacillus subtilis and Bacillus cereus, which cause spoiling of cooked rice and bread, as representative gram-positive bacteria, and against Escherichia coli, which is an index of general contamination, as a representative gram-negative bacterium. The results are shown in the table.

[0053] An amount of 1 ml of the product of this invention was added to 10 ml of ordinary agar culture medium as the culture medium. Culture medium to which 1 ml of water was added instead of the product of this invention was used as the control. Culturing was performed for 48 hours at 37°C and the state of growth of each bacterium was observed. The results are shown in Table 6.

[0054]

[Table 6]

	Red bean (azuki] aqueous extract	Soybean aqueous extract	Water
Bacillus subtilis	–	–	+++
Bacillus cereus	–	–	+++
Escherichia coli	–	+	+++

Note 1 Evaluations: - : no growth; +: small amount of growth; ++ growth; +++: large amount of growth

Note 2 The red bean aqueous extract used was that obtained in Example 1 and the soybean aqueous extract used was that obtained in Example 2.

[0555] As should be evident from Table 6, in the culture media in which water was added as a control there was fairly extensive growth of both food spoilage bacteria and Escherichia coli on culturing for 48 hours at 35°C. By contrast, although there was slight growth of Escherichia coli in culture media to which the product of this invention was added, no growth whatsoever of bacteria of the genus Bacillus was found. From these results, it was ascertained that products of this invention have extremely strong antibacterial effects.

[0056] Next, a study was made of the effects of the products of this invention in inhibiting formation of oxides by the iron rhodanide method. Specifically, a study was made of the effects of the products of this invention in inhibiting oxidation of linoleic acid, which is extremely readily oxidized. The determination method is described below.

[0057] Preparation of reagents

1. 0.2 M phosphate buffer solution (pH 7.0)

2. 2.6% ethanol lineoleate solution

3. 75% ethanol solution

4. 30% ammonium thiocyanate

5. 35% hydrochloric acid solution of 0.02 M of ferric chloride

[0058] Operational methods

1. 0.1 ml of test material solution, 0.1 ml of 0.2 M phosphate buffer solution, 0.5 ml of water and 0.2 ml of 2.6% ethanol lineolate solution were added and were thoroughly mixed, after which the mixture was allowed to stand for 5 days at 37°C.

2. 50 µl of oxidation treatment solution , 4.85 ml of 75% ethanol solution, 50 µl of 30% ammonium thiocyanate and 50 µl of 35% hydrochloric acid solution of 0.02 M ferric chloride solution were mixed and the absorbance of the mixture at 500 nm was determined after 5 minutes.

3. A blank was made by the same procedure using distilled water instead of the sample. The results are shown in Table 7.

[0059]

[Table 7]

Name of Sample	A_{500}	Oxidation percentage (%)
Water (control)	0.352	100.0
Red bean aqueous extract	0.064	18.2
Soybean aqueous extract	0.047	13.4

Note 1 The red bean aqueous extract used was the product of this invention that was obtained in Example 1

Note 2 The soybean aqueous extract used was the product of this invention that was obtained in Example 2.

[0060] As should be evident from Table 7, it was ascertained that the product of this invention has a superior antioxidant effect for lineoleic acid, which is extremely easily oxidized.

[0061] Thus, because the products of this invention are safe for human beings, have antibacterial effects against diverse bacteria, have a browning preventing effect and have an antioxidant effect, they can be widely used for food products as preservatives and as antioxidants and agents for maintaining freshness.

[0062]

[Effect of the invention] As should be evident from the data described above, active oxygen elimination agents that are simple, completely safe and stable, that have a superior active oxygen elimination effect and that have other diverse effects can be obtained by subjecting legumes to aqueous extraction, organic solvent extraction or squeezing.

[0063] Because legumes have long been used as foods, almost no methods of manufacture or uses have been developed in new fields apart from their use as foods. Further, because legumes have been used as foods, their safety has been demonstrated.

[0064] Consequently, it was discovered that this invention can be used as a medicinal drug product for the prevention or treatment of the diseases indicated above, that it can be added to foods and cosmetic products and can play a role in promoting health and for purposes of beauty and that active oxygen elimination agents that can be used in a broad range of fields can be obtained simply from familiar legumes the safety of which has been demonstrated.

[0066] Up to the present, legumes have been used only as foods. The fact that mew uses for legumes have been developed and that the new possibilities have been discovered for them is of extremely great significance.

[0066]

[Examples] Example 1

1 kg of mature dry red beans was thoroughly pulverized, 3 liters of warm water at 55°C and 15 g of liquefaction enzyme were added to them and the mixture was thoroughly stirred. Following that, it was gradually heated and extraction by boiling was performed for 5 minutes, after which it was cooled to 30°C. Following that, it was squeezed with a squeezing machine, with 2.5 liters of red bean aqueous extraction solution and 1.4 kg of residue being obtained.

[0067] Example 2

1 kg of mature soybeans was thoroughly pulverized, 2 liters of 90% alcohol were added, the mixture was thoroughly stirred and was then allowed to stand for 24 hours, after which it was squeezed with a squeezing machine, with 1.5 liters of pressed solution and 1.3 kg of residue being obtained. 2.0 liters of water were added to the pressed solution, it was concentrated under reduced pressure, the ethanol was removed and 1.4 liters of product of this invention were obtained.

[0068] Example 3

3 kg of green soybeans were introduced into a pressing machine and 0.6 liter of juice and 2.4 kg of residue were obtained.

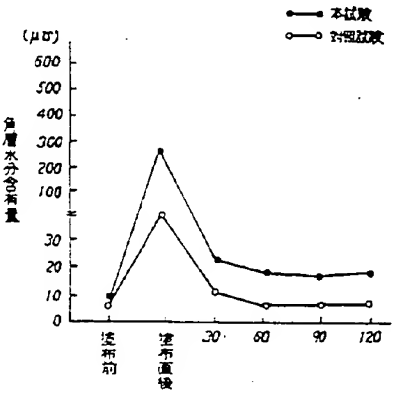
[Brief Explanation of the Figures]

[Figure 1] This is a graph that shows the results for the moisture maintaining effect of a product of this invention and water when single application experiments were performed using a moisture meter (SKICON 200).

[Figure 2] This is a graph that shows the results when water load experiments were performed before use of the product of this invention and after two weeks of use.

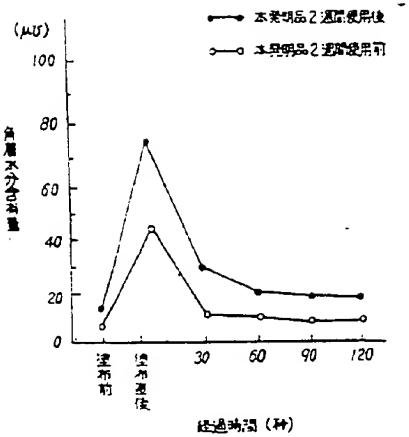
[Figure 3] This is a graph that shows the results of experiments on changes of sebum content in cases in which the product of this invention was applied after washing of the face and in cases of face washing only.

[Figure 1]



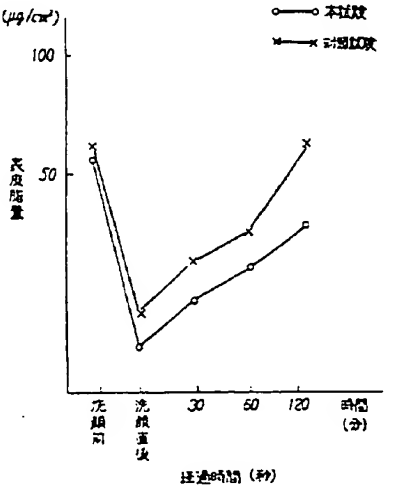
[vertical axis]: Water content of stratum corneum
[horizontal axis: horizontal characters]: Course over time (minutes)
[horizontal axis: vertically written character groups, left to right]:
Before application; Immediately after application
[inside graph at upper right]
This experiment
Control experiment

[Figure 2]



[vertical axis]: Water content of stratum corneum
[horizontal axis: horizontal characters]: Course over time (seconds)
[horizontal axis: vertically written character groups, left to right]:
Before application; Immediately after application
[inside graph at upper right]
After 2 weeks of use of product of this invention
Before 2 week use of product of this invention

[Figure 3]



[vertical axis]: Amount of sebum in epidermis
[horizontal axis: horizontal characters]: Course over time (seconds)
[horizontal axis: vertically written character groups, left to right]:
Before washing face; Immediately after washing face
[inside graph at upper right]
This experiment; Control experiment

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号
特開平5－320061
(43)公開日　平成５年(1993)12月３日

(51)Int.Cl.[5]	識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ａ６１Ｋ　35/78	ＡＤＤ　Ｊ	7180－4Ｃ		
	ＡＣＬ	7180－4Ｃ		
	ＡＤＡ	7180－4Ｃ		
	ＡＥＤ	7180－4Ｃ		
7/00	Ｋ	9165－4Ｃ		

審査請求　未請求　請求項の数１（全　９　頁）　最終頁に続く

(21)出願番号　特願平4－150155
(22)出願日　平成４年(1992)５月19日

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(54)【発明の名称】　豆類からの活性酸素消去剤

(57)【要約】
【目的】　豆類を原料として、安全で安価で、医薬、食品、化粧品等幅広い分野で使用可能な活性酸素消去剤を提供する。
【構成】　豆類からの水抽出物または有機溶媒抽出物あるいは生豆からの搾汁液をそのまま、あるいはこれを含有してなることを特徴とする活性酸素消去剤。

1

【特許請求の範囲】

【請求項1】　豆類からの水抽出物または有機溶媒抽出物あるいは生豆からの搾汁液をそのまま、あるいはこれを含有してなることを特徴とする活性酸素消去剤。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、豆類からの水抽出物または有機溶媒抽出物あるいは生豆の搾汁液をそのまま、あるいはこれらを含有してなることを特徴とする安全で安価で、医薬、食品、化粧品等幅広い分野で使用可能な活性酸素消去剤に関するものである。

【0002】

【従来の技術】人間が健康体を保っている場合、生体内の活性酸素と生体内での活性酸素消去酵素であるSOD（スーパーオキサイドジスムターゼ）は、常にバランスがとれており、活性酸素の濃度は、ほぼ一定に保たれている。しかし、現在では、食生活のアンバランス、過度のストレスおよび高齢化などにより、SODの生成が減少し、また、一方では、喫煙、大気汚染などにより、活性酸素が増加している。

【0003】その結果、生体内に活性酸素が過剰に存在し、様々な組織障害をもたらしている。特に高齢者の場合、SOD活性が低下し、活性酸素濃度が高くなることにより、関節リウマチやベーチェット病などの障害を起こしている。また、活性酸素により生成する過酸化脂質は、心筋梗塞、脳卒中、白内障、シミ、ソバカス、皺、糖尿病、動脈硬化、肩凝り、冷え性などの近代病の主原因となっている。

【0004】また、高齢者でなくても、皮膚のように紫外線などのような環境因子の刺激を直接受ける部位では、活性酸素が特に生成しやすいため、活性酸素濃度の上昇にともない、メラニン色素の生成、シミ、小皺等の障害を起こしやすくなっている。

【0005】そこで、上述のような各種障害のもととなる過剰な活性酸素を消去するSODが注目をあび、これらの障害を予防または治療するために、SODを医薬品としたり、化粧品や食品に添加したりして利用する試みは行われてきた。しかし、SODは熱に不安定であり、しかも、経口投与により失活してしまうため、また、著しく高価であるため、SODによる活性酸素の消去は未だ成功していない。

【0006】上記実情から、活性酸素消去剤（SOD酵素と同じような働きをする抗酸化物質を含むもの）の研究が行われ、生薬抽出エキス等による活性酸素消去剤も開発されているが、特殊な原料によるものであり、高価であるばかりでなく、なかなか安定したものを供給することができないのが現状である。

【0007】

【発明が解決しようとする課題】以上のように活性酸素による各種の障害が認められて以来、生体内の活性酸素を消去するためのさまざまな研究が盛んに行われている。また、現在では、高齢化社会を迎えて、より健康で老後をすごすということが望まれている。一方、美容の面からも、活性酸素消去剤が注目をあびている。

2

【0008】そこで、人体にとって安全で安価で、各種障害を起こす活性酸素の消去効果に優れ、しかも、簡単に製造でき、安定して供給できる活性酸素消去剤の開発が望まれている。

【0009】

【課題を解決するための手段】本発明者らは、動植物合和すの観点から、種々の植物成分の研究を進めてきた。その過程で大豆、えんどう豆、いんげん豆、小豆、そら豆等で代表される豆類には、今まで予測できなかった数多くの可能性、効果があることが判明してきた。そこで、古くから世界中で食用として用いられ、安全性が最も高いことが実証されている豆類をテーマとして取り上げ、豆類の総合利用研究を行ってきた。そのうちの一つのテーマとして、豆類からの活性酸素消去剤について鋭意研究を行い、豆類の抽出物または搾汁液をそのまま、あるいはこれを含有するものの活性酸素消去効果を測定したところ、非常に顕著な活性酸素消去効果があることが判明し、本発明を完成するに至った。

【0010】すなわち、本発明は、豆類の水抽出物または有機溶媒抽出物あるいは豆の搾汁液をそのまま、あるいはこれを含有してなることを特徴とする活性酸素消去剤であって、豆を圧搾あるいは水抽出（酸、アルカリ抽出も含む）またはアルコールなどの有機溶媒で抽出することにより、簡単、安価に、しかも、全く安全に、上記の効果を顕す非常に優れた活性酸素消去剤が得られるのである。ここで原料として用いる豆類は、大豆、えんどう豆、いんげん豆、小豆、そら豆等どんな種類の豆でもよい。また、豆の状態は、未熟生豆、完熟生豆、さらに乾燥処理した完熟豆等どんなものでもよい。

【0011】豆を水抽出または有機溶媒抽出する場合、まず、豆を粉砕または粉体化すると表面積が大きくなるため、極めて抽出効率が良好になる。この方法は、粉砕機を用い、一般的な方法によればよい。粉砕しなくてもよいが、この場合には、豆組織の分解および抽出に長時間を要する。

【0012】水抽出に当たっては、豆をそのまま、好ましくは粉砕または粉体化したものに加水する。加水量については、豆に対して2～5倍量で効率よく抽出されるが、収率、作業性、最終使用目的等に応じて適宜選定すればよい。この後加温してゆき、沸騰状態になった時点で抽出を完了する。抽出を完了した後、使用目的により圧搾、濾過を行えば、清澄な抽出エキスが得られる。なお、最初から熱水を加えて抽出を行ってもよい。

【0013】抽出液中の有効成分は解明されていないが、この未知の有効成分が熱に安定であることは確認できたので、水抽出の際の抽出温度は、高温が効率的であ

も，低温でも長時間置けば，充分に抽出を行うことができる。ただし，４０℃以下の低温の場合は，ｐＨを酸性あるいはアルカリ性にするか，防腐剤を加えることが必要である。抽出時間は，沸騰抽出の場合には数分でよいが，それ以下の中温の場合には，数時間から一昼夜が必要である。低温の場合は，豆の粉砕状態にもよるが，数日～１ケ月必要である。ただし，この場合にも，なるべく最後には加熱するのがより効果的である。

【００１４】澱粉主体の成分の豆の水抽出の場合に最も問題になるのは，糊化現象である。糊状になれば抽出効率が悪くなるのみでなく，実作業においては困難を極める。これを防ぐためには，アミラーゼを加えて反応させるか，塩酸などで酸性にして澱粉を分解すればよく，この方法を用いることにより，充分に解決でき，実用上も全く問題がない。

【００１５】抽出液中の有効成分は，酸，アルカリに安定であるためか，酸抽出あるいはアルカリ抽出を行うのも有効である。また，水抽出の場合，酸，アルカリで前処理するか，豆の組織に働く酵素（例えば，セルラーゼ，リパーゼ）を反応させて前処理を行い，抽出する方法が効率的である。これは，前処理により，有効成分がより抽出されやすくなるためであると思われる。

【００１６】さらに，有機溶媒抽出でも，本効果をもったエキスが抽出されることが判明した。このことは，有効成分の解明を進める上で，また，有効成分をコンクに抽出したり，水に溶けないものとの配合という利用用途の上で極めて有効である。この場合，なるべく微粉砕または粉体化することが好ましい。また，ここで用いる有機溶媒はアルコールのような人体に投与しても安全なものを使用することが望ましい。

【００１７】また，生豆からの有効成分の採集には，豆の搾汁をとるのも有効であった。豆の搾汁をとる場合には，未熟生豆や完熟生豆，さらにはそれらを加熱処理したものを磨砕し，布袋に包んで絞るか圧搾機等を用いて搾るなど，一般的な方法によればよい。この場合にも，圧搾をした後の残渣を，さらに，水抽出または有機溶媒抽出することにより，効果としては弱いが，活性酸素消去作用があることも判明した。

【００１８】さらに，アルコール発酵，乳酸発酵等の発酵を組み合わせても同等の効果であった。なお，本発明品の豆類からの抽出には，以上のように有機溶媒抽出または水抽出し，その抽出物中の有効成分をさらに溶媒抽出すると，より有効である。しかし，これは，濃縮状態が得られるためと思われ，濃縮することにより同等の効果が得られる。

【００１９】また，用途によっては搾汁や水抽出物に糖やデキストリンが含まれてベタつくとか，その効果において邪魔になることがある。その場合には，糖を酵母に食べさせるとか，有効成分を吸着剤で分画するとか，有機溶媒で抽出することにより糖を除いてやればよい。いずれにしても，抽出さえ行えば効果が出てくるわけで，用途によっては不要の成分は種々の方法により取り除けばよい。

【００２０】豆類は古くから食用として毎日用いられており，あまりにも身近すぎて，このように活性酸素消去剤として使用する概念すらなく，思いもよらないことであった。また，豆の形で食べる以外には，豆腐，湯葉，油揚，餡，黄粉，納豆，味噌，醤油等，いろいろな形に加工されて用いられてきたが，豆類の抽出という考え方も方法も取られていなかった。これは，加熱抽出しようとすると，澱粉豆の場合，糊状になり，従来の考え方では非常に困難であったことにもよるものと思われる。そのため，本発明においては，有機溶媒抽出，酸，アルカリ抽出を用い，また，水抽出の場合，アミラーゼなどを作用させ，抽出を容易にすることにより，目的を達成することができるようにしたのである。

【００２１】また，大豆などのように蛋白質が主成分の豆の場合には，従来から抽出して豆乳を取る方法も用いられてきたが，栄養源としての利用のみであり，活性酸素消去剤としての利用は全くなされていなかった。また，従来の豆乳には，ほとんど活性酸素消去作用は認められなかった。すなわち，前処理としてセルラーゼ，リパーゼ等の豆の組織に働く酵素による処理，または酸，アルカリによる処理を行うことによりはじめて，目的を達成することができるようにしたのである。このように原料に合わせて充分抽出操作を行って初めて，非常に優れた活性酸素消去剤としての有効成分を抽出することができるのである。

【００２２】本発明品の活性酸素消去効果について，以下に記載する。まず，各種イモ操作方法によるスーパーオキサイド消去剤としての効果を調べた。試験方法はＮＢＴ法により行った。

【００２３】試薬の調整

① ０．０５Ｍ Na_2CO_3 緩衝液（ｐＨ１０．２）

② ３ｍＭキサンテン溶液；キサンテン４５．６４㎎を①の緩衝液に溶解して１００mlとする。

③ ３ｍＭ　ＥＤＴＡ溶液；ＥＤＴＡ・２Ｎａ　１１１．７㎎を蒸留水で溶解して１００mlとする。

④ ＢＳＡ溶液；Bovine Serum Albumin (Sigma 製) １５㎎を蒸留水に溶解して１０mlとする。

⑤ ０．７５ｍＭ　ＮＢＴ溶液；ＮＢＴ（ニトロブルーテトラゾリウム）６１．３２㎎を蒸留水に溶解して１００mlとする。

⑥ キサンテンオキシダーゼ溶液；キサンチンオキシダーゼを蒸留水で希釈し，後記の操作法（分析法）の空試験における吸光度が０．２～０．２３の範囲になるように調整する。

⑦ ６ｍＭ　$CuCl_2$ 溶液；$CuCl_2 \cdot 2H_2O$ １０２．２９㎎を蒸留水に溶解して１００mlとする。

【００２４】操作法

5　　6

① 試験管にNa₂CO₃緩衝液2．4mlをとり、これにキサンテン溶液、EDTA溶液、BSA溶液、NBT溶液を各0．1ml加える。

② 次いで、試料溶液0．1mlを加え、25℃で10分間放置後、キサンテンオキシダーゼ溶液0．1mlを加え、手早く撹拌し、25℃でインキュベートする。

③ 20分後にCuCl₂溶液0．1mlを加えて反応を停止させ、560nmで吸光度を測定する。

④ 比較のため、サンプルの代わりにスーパーオキサイ＊ドジスムターゼ（Cu．Zn型SOD、活性3000～4000 unit／mg 和光純薬）水溶液0．1mlについても同様に行い、この値をスーパーオキサイド消去率100とする。

⑤ また、サンプルの代わりに蒸留水を用いて同様に行いブランクとする。測定結果を表1に示した。

【0025】

【表1】

	小豆		蚕豆		大豆		SOD
	水抽出物	有機溶媒抽出物	水抽出物	有機溶媒抽出物	水抽出物	有機溶媒抽出物	
スーパーオキサイド(SO) 消去率（%）	88	95	89	63	66	50	100

注1　小豆水抽出物は実施例1により得られた本発明品を用いた。

注2　大豆有機溶媒抽出物は実施例2により得られた本発明品を用いた。

【0026】以上のように、水抽出物においても有機溶媒抽出物においても、スーパーオキサイド消去効果があることが分かった。さらに、その効果は、アルコール発酵、乳酸発酵を行ってもほとんど同様の効果であること※が判明した。

【0027】次に、本発明品の熱安定性について調べた。まず、実施例1により得られた本発明品およびSODを90℃10分間加熱処理し、そのスーパーオキサイド消去能を調べた。スーパーオキサイド消去率の測定は、前記方法により行った。その結果を表2に示した。

【0028】

【表2】

	小豆水抽出物	蚕豆水抽出物	大豆水抽出物	SOD
SO消去率（%）	89	88	65	0

注　表1と同様、各抽出物は各実施例により得られたものを使用した。

【0029】以上のように、SODは熱に対して不安定なのに対して、本発明品は全て熱安定性に優れていることが分かった。このことより、本発明品の活性酸素を消去する有効成分は、熱に対しても安定性に優れているといえる。本発明品は、非常に顕著な活性酸素消去効果を示し、しかも、安全なものであるから、医薬、化粧品、食品などに利用できるものである。次に、これらの用途について説明する。

【0030】医薬品としては、抗潰瘍剤として利用できる。本発明品の抗潰瘍作用について調べた試験方法とその結果について示すと、次のとおりである。拘束水浸ストレス潰瘍に対する本発明品の経口投与においての作用を調べた。その方法は、渡辺らの方法に準じて行った。すなわち、8週齢のddY系雄性マウスを24時間絶食後、実施例1により得た本発明品を0．3ml／マウス経口投与し、30分後にストレスゲージに入れ、15℃の水中に剣状突起まで浸し、拘束水浸ストレスを負荷した。5時間後に頸椎脱臼して屠殺し、胃を摘出した。その後、1％ホルマリン溶液1．5mlを胃内に注入し、さらに、同液中に浸すことにより胃組織を軽く固定し、24時間そのまま放置した。その後、大弯に添って切開し、腺胃部に発生した損傷の長さ（mm）を測定し、一匹当りのその総和を潰瘍係数として表した。また、コントロールとしては、ストレスゲージに入れる30分前に同量の生理食塩水を経口投与したものを用いた。マウスは各々15匹ずつで行った。その結果を示すと表3のとおりである。

【0031】

【表3】

	投与量（ml／マウス）	検体数	潰瘍係数の平均
生理食塩水	0．3	15	65．8
本発明品	0．3	15	34．9

【0032】表3のように、コントロールとして生理食塩水を投与したマウスにおける潰瘍係数の平均が65．8であるのに対して、本発明品を投与したマウスにおける潰瘍係数の平均は34．9となり、明らかに本発明品は、経口投与することにより拘束水浸ストレス潰瘍に対する抗潰瘍剤として有効であることが判明した。この結果、本発明品は、胃腸粘膜から直接に作用して抗潰瘍剤＊として有効な作用を示すことが判明した。

【0033】次に、本発明品は、皮膚治療剤として利用できる。各種皮膚疾患のパネラーに、本発明品を毎日、朝、晩2回患部に塗布させ、これを1ケ月間継続して行い診断した結果を表4に示した。

【0034】

【表4】

	著名改善	有用	やや有用	どちらともいえない	中止	有用率（%）
すり傷、きり傷	0	3	5	4	0	66.7
火　傷	1	3	5	2	0	81.8
おむつかぶれ	0	3	6	2	0	81.8
虫ささされ	0	1	4	2	0	71.4
おでき、吹出物	0	5	9	4	0	77.8
にきび	2	3	5	2	0	83.3
ひび、あかぎれ	0	3	3	2	0	75.0
乾皮症	1	2	8	2	0	84.6
皮膚のかゆみ	0	2	12	5	0	73.7
湿　疹	1	4	6	3	0	78.6
アトピー性皮膚炎	1	5	4	6	0	62.5
小水泡型水虫	0	3	4	1	0	87.5
角化型水虫	0	4	2	1	0	85.7

（注）1　本発明品は実施例1により得られたものを使用した。
（注）2　有用率は著名改善＋有用＋やや有用の全体の割合
（注）3　判定は専門の医師により行った。
（注）4　パネラーは男性35名、女性33名　計68名
平均年齢32．5歳（年齢1～79歳）であった。

【0035】上記の表4に示すように、本製品にはさまざまな皮膚治療剤としての効果があることから、繊維芽細胞賦活作用、さらには抗菌作用があることが分かる。また、乾皮症、にきび等にも有用なことから、保湿作用、脂皮の増大を適度に抑制する作用もあることが分かるが、実際にこの保湿作用および脂皮の増大を適度に抑制する作用について調べた試験方法とその結果を示すと、次のとおりである。

【0036】まず、本発明品の保湿作用の強さを例証するために、水分計（SKICON200）を用いて1回塗布試験を行った。測定条件として室温20℃、相対湿度65％の環境を設定し、パネラーには測定の約10分前から、前記の環境下で安静にさせておいた。被験部位は（両側）前腕屈側で皮疹の認められていない部位を選ん

だ。パネラーは乾皮症で悩んでいる5名で行った。水分計から読み取った本試験（実施例1により得られた本発明品を用いた）と対照試験（水使用）との角層水分含有量の変化の平均値を図1に示した。1回塗布試験の測定方法は下記のとおりである。

【0037】測定方法

1）パネラーの前腕屈側に5×5cmの被験部位と対照部位を設定する。

2）それぞれの部位の角層水分含有量を測定する。

3）試料塗布直後、30、60、90、120分後の角層水分含有量を測定する。

【0038】図1から、本発明品は、塗布直後、角層水分含有量において、対照の約8倍ほどの増加が認められた。また、塗布後30分から120分までについてみると、本発明品塗布部位では、120分まで対照の2～3倍の水分を維持していることが分かる。

【0039】次に、本発明品の乾皮症の治療効果を数値的に実証するために、水分計（SKICON200）を用いて本発明品使用前と2週間使用後の水負荷試験を行った。パネラーは図1で使用した5名を用いて行い、測定条件も1回塗布試験と同一条件下で行った。なお、効果判定に季節的な生体角層の水分含量の変化が影響しないように必ず対照（本発明品無塗布部位での測定）をおくようにした。角層水分含有量はパネラー5名の平均値で示した。この結果を図2に示した。本発明品は、実施例1により得られたものを用いた。また、水負荷試験の測定方法は下記のとおりである。

【0040】測定方法

1）被験部位の角層水分量を測定する。

2）蒸留水を1滴被験部位にのせ、10秒後に乾いたガーゼで水滴を完全に拭きとる。

3）拭きとった直後、30、60、90、120秒後の角層水分含有量を測定する。

【0041】図2のグラフが示すように、本発明品塗布により、皮膚の水分吸水能（水負荷後0秒の角層水分含有量から負荷前の角層水分含有量の値を引いたもの）、水分保持能（水負荷後0秒から120秒までの角層水分含有量の描く曲線）の双方を同時に改善させていることが分かる。

【0042】すなわち、本発明品使用前の皮膚は、水負荷前の角層水分含有量が非常に低く（平均4．6）、吸水能（平均42．0）もかなり低下している。また、水分保持能も正常人の皮膚の角層は、吸水した水分を徐々に放出していくのに比べ、水負荷30秒後には、水負荷前の値に戻ってしまっている。これらの結果は、測定した病的角層においては、吸水能、水分保持能、バリア機能すべてが低下していることを物語っている。これに対し、本発明品使用後の皮膚は、水負荷前の角層水分含有量も吸水能も約2倍に増え、水分保持能も正常人と変わらないほどにかなり改善されていることが分かった。

【0043】このことから、本発明品は、病的角層の水分含有状態やバリア機能改善について優れた作用があるといえる。また、1回塗布試験より得た保湿作用と合わせて本発明品を評価すると、本発明品は、角層の吸水能、水分保持能を増大し、水分を外界から多く吸収し、さらに、一度吸収した水分を放さないようにする性質を角層に与えるという保湿作用があるといえる。

【0044】さらに、本発明品の皮脂量の分泌抑制効果を実験的に例証するために、洗顔後の皮脂量の変化を測定した。パネラーは表4で使用した中から無作為に選んだ5名を用い、本試験（洗顔後、本発明品を塗布）と対照試験（洗顔のみ）との皮脂量の変化の平均値を図3に示した。なお、本発明品は、実施例1により得られたものを用いた。

【0045】図3のグラフに示すように、本発明品を塗布すると皮脂量の増大がかなり抑制されることが判明した。この本発明品の皮脂量分泌抑制効果からも、ニキビの予防治療効果が裏付けられた。また、本発明品を化粧品として肌に塗布すると、肌がつるつるする、きめが細かくなるという効果、しわがのび若返る効果、さらに老化防止効果があることが、次の試験から明らかになった。

【0046】本発明品をパネラーの右腕部位に1日2回1ケ月間塗布させ、本発明品塗布部位を動摩擦計で測定した。対照は左腕の同部位を用いた。パネラーは6名で行った。

測定条件は下記のとおりである。

温　度　　25℃
湿　度　　60％
使用センサー　KES－SE摩擦感テスターSE－2タイプ（0．5mmピアノワイヤー使用）
摩擦静荷重　　50gf
測定速度　　1mm／sec
測定距離　　30mm（積分有効範囲20mm）

【0047】本発明品を塗布していない左腕の部位では、MMD（変動係数）0．0186であったのが、本発明品を1ケ月間塗布した右腕の部位では、、MMD（変動係数）0．0084に下がった。6名の平均値もほぼ同様であった。これは、表面の凹凸による変動が小さくなったためと考えられ、このことから、肌のきめが細かくなったこと、さらには、しわがのび若がえることが判明した。なお、同時にMIU（摩擦係数）も調べたところ、塗布前は0．138であったのが、1ケ月塗布後の肌は0．102に下がり、肌をつるつるさせる効果、肌をやわらかくする効果、さらには、老化防止効果をも合わせ持つことが判明した。

【0048】さらに、本発明の美白作用を例証するために、テロシナーゼ活性阻害作用の試験を行った。操作方法としては、基質液（0．04％テロシン溶液）、緩衝液（McIlvaineBuffer　pH6．8）各1mlを吸光セル

に正確に取り、水および実施例１で得られた本発明品を、それぞれ１mlづつ正確に入れ、撹拌混和して３５℃に保ち、５分後、吸光度目盛を波長４７５nmに合わせてゼロ補正を行い、次いで、テロシナーゼ溶液（テロシナーゼ５．３mgを０．９％ＮａＣｌ溶液に溶かしたもの）０．０２mlを正確に加え、直ちに撹拌してインキュベートした。この時の吸光度を経時間（３分置き）に測定し、表５に示した。

【００４９】

【表５】

分	水	本発明品
0	0．011	0．012
3	0．058	0．045
6	0．152	0．059
9	0．243	0．097
12	0．316	0．099
15	0．414	0．118
18	0．498	0．133
21	0．552	0．142
24	0．621	0．151
27	0．623	0．157
30	0．629	0．162

【００５０】表５に示す測定結果から、本発明品は、チ＊ロシナーゼ活性阻害作用を有することが分かる。このことから、本発明品には美白作用があるといえる。

【００５１】さらに、前記に述べたように、本発明品は、医薬品として使用できるほどの保湿作用も持っている。したがって、化粧品の基本となる作用を全て満足していることになり、クリーム、乳液、化粧水、クレンジング、パック、石けん等、幅広い利用用途がある。また、本発明品を飲用することによっても、上記と同様の効果が得られた。

【００５２】本発明品は、食品の保存剤、鮮度保持剤としても利用できるものである。次に、グラム陽性菌の代表として、米飯やパンなどの腐敗を起こす Bacillussubtilis、Bacillus cereus 、およびグラム陰性菌の代表として、一般的な汚染の指標とされている大腸菌 Escherichia coli に対する本発明品の抗菌力試験と、その結果を示す。

【００５３】培地は、普通寒天培地１０mlに、本発明品１ml添加したものを用いた。コントロールとして、本発明品の代わりに水１mlを添加したものを用いた。培養は３７℃４８時間行ない、各菌の発育状態を観察し、表６に示した。

【００５４】

【表６】

	小豆水抽出物	大豆水抽出物	水
Bacillus subtilis	－	－	+++
Bacillus cereus	－	－	+++
Escherichia coli	－	＋	+++

注１　評価は　－：発育せず　＋：少し発育あり
＋＋：発育あり　＋＋＋：発育大
注２　小豆水抽出物は実施例１により得られたもの、大豆水抽出物は実施例２により得られたものを用いた。

【００５５】表６から明らかなように、コントロールとして水を添加した培地では、３５℃で４８時間培養において、食品の腐敗菌も大腸菌もかなり発育が大きかったのに対して、本発明品を添加した培地では、大腸菌はわずかに発育が認められたものの、Bacillus 属の菌の発育はまったく認められなかった。この結果より、本発明

品は、きわめて有効な抗菌効果を有するものであることが判明した。

【0056】次に、本発明品による酸化物の生成抑制効果をロダン鉄法により調べた。すなわち、本発明品によるきわめて酸化されやすいリノール酸の酸化抑制効果を調べた。測定方法は以下に示すとおりである。

【0057】試薬の調製

① 0．2M リン酸緩衝液（pH7．0）
② 2．6% リノール酸エタノール溶液
③ 75% エタノール溶液
④ 30% アンモニウムチオシアネート
⑤ 0．02M 塩化第二鉄の35%塩酸溶液

【0058】操作方法

*① 試料溶液0．2ml、0．2Mリン酸緩衝液0．1ml、水0．5ml、2．6%リノール酸エタノール溶液0．2mlを加えてよく混合し、37℃で5日間放置する。

② ①の酸化処理液50μl、75%エタノール溶液4．85ml、30%アンモニウムチオシアネート50μl、0．02M塩化第二鉄の35%塩酸溶液50μlを混合し、5分後に500nmの吸光度を測定する。

③ また、サンプルの代わりに蒸留水を用いて同様に行い、ブランクとする。結果は表7に示した。

【0059】

【表7】

サンプル名	A_{500}	酸化割合（%）
水（コントロール）	0．352	100．0
小豆水抽出物	0．064	18．2
大豆水抽出物	0．047	13．4

注1 小豆水抽出物は実施例1により得られた本発明品を用いた。

注2 大豆水抽出物は実施例2により得られた本発明品を用いた。

【0060】表7から明らかなように、本発明品は、きわめて酸化されやすいリノール酸に対して、優れた酸化防止効果を持つことが判明した。

【0061】このように、本発明品は、人体に対して安全でさまざまな菌に対する抗菌効果、褐変防止効果、さらには抗酸化効果を有することから、保存料さらには抗酸化鮮度保持剤として、広く食品に用いることができるものである。

【0062】

【発明の効果】前記のデーターからも明らかなように、豆類を水抽出あるいは有機溶媒抽出あるいは搾汁することにより、簡単に、全く安全で、熱に対して安定で、しかも、活性酸素消去効果に優れ、さらに、さまざまな効果を有する活性酸素消去剤が得られる。

【0063】豆類は古くから食用として用いられてきたため、食以外の新規な分野での製法、利用用途はほとんど開発されていなかった。さらに、豆類は食として用いられてきたものであり、安全性も実証されているものである。

【0064】したがって、本発明は、前述の疾患の予防ないし治療のための医薬品として使用できるほか、食品、化粧品等に添加して健康増進、美容のために役立たせることも可能であり、幅広い分野で利用可能な活性酸素の消去剤を、安全性の実証されている身近な豆類から簡単に得られることを見出したものである。

【0065】また、豆は今まで食としての利用しかなされていなかったものであり、豆の新たな利用用途を開発し、新たな可能性を見出したことは極めて有意義なことである。

【0066】

【実施例】実施例1

完熟乾燥小豆1kgをよく粉砕し、これに55℃の温水3リットルと液化酵素15gを加え、よく撹拌した。その後、徐々に加温してゆき、5分間煮沸抽出した後、30℃まで冷却した。その後、しぼり機でしぼり、小豆水抽出液2．5リットルと残査1．4kgを得た。

【0067】実施例2

完熟乾燥大豆1kgをよく粉砕し、90%アルコール2リットルを加え、よく撹拌して24時間放置した後、しぼり機でしぼり、圧搾液1．5リットルと残渣1．3kgを得た。この圧搾液に2．0リットル加水し、減圧下で濃縮してエタノールを除去し、本発明品1．4リットルを得た。

【0068】実施例3

枝豆3kgを圧搾機にかけ、搾汁液0．6リットルと残渣2．4kgを得た。

【図面の簡単な説明】

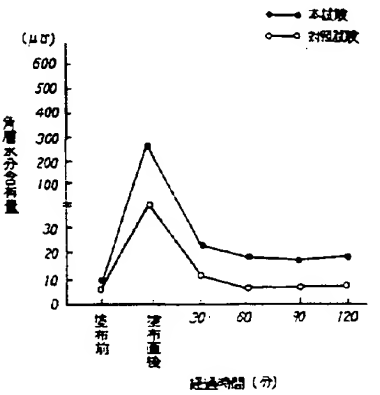
【図1】本発明品と水の保湿効果について、水分計（SKICON200）を用い1回塗布試験を行った結果を示すグラフである。

【図2】本発明品使用前と2週間使用後の水負荷試験を行った結果を示すグラフである。

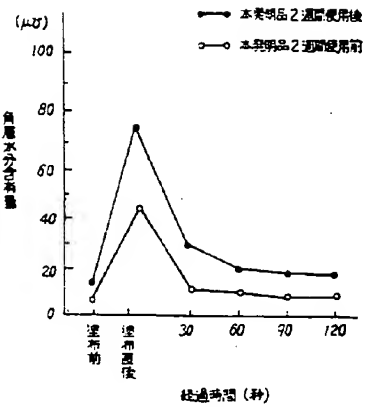
15　　16

【図３】洗顔後に本発明品を塗布した場合と洗顔のみの　　である。
場合の皮脂量の変化について試験した結果を示すグラフ

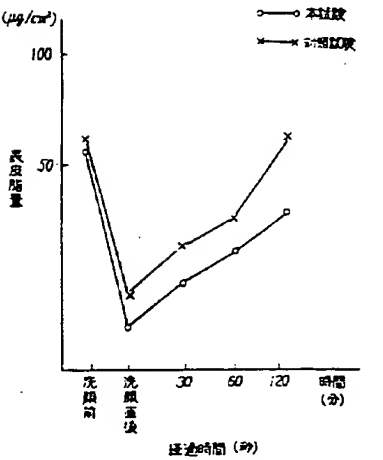
【図１】



【図２】



【図３】



フロントページの続き

(51)Int.Cl.⁵		識別記号	庁内整理番号	FI	技術表示箇所
A61K	7/00		X 9165－4C		
			Y 9165－4C		
	7/48		9051 －4C		
C09K	15/34				